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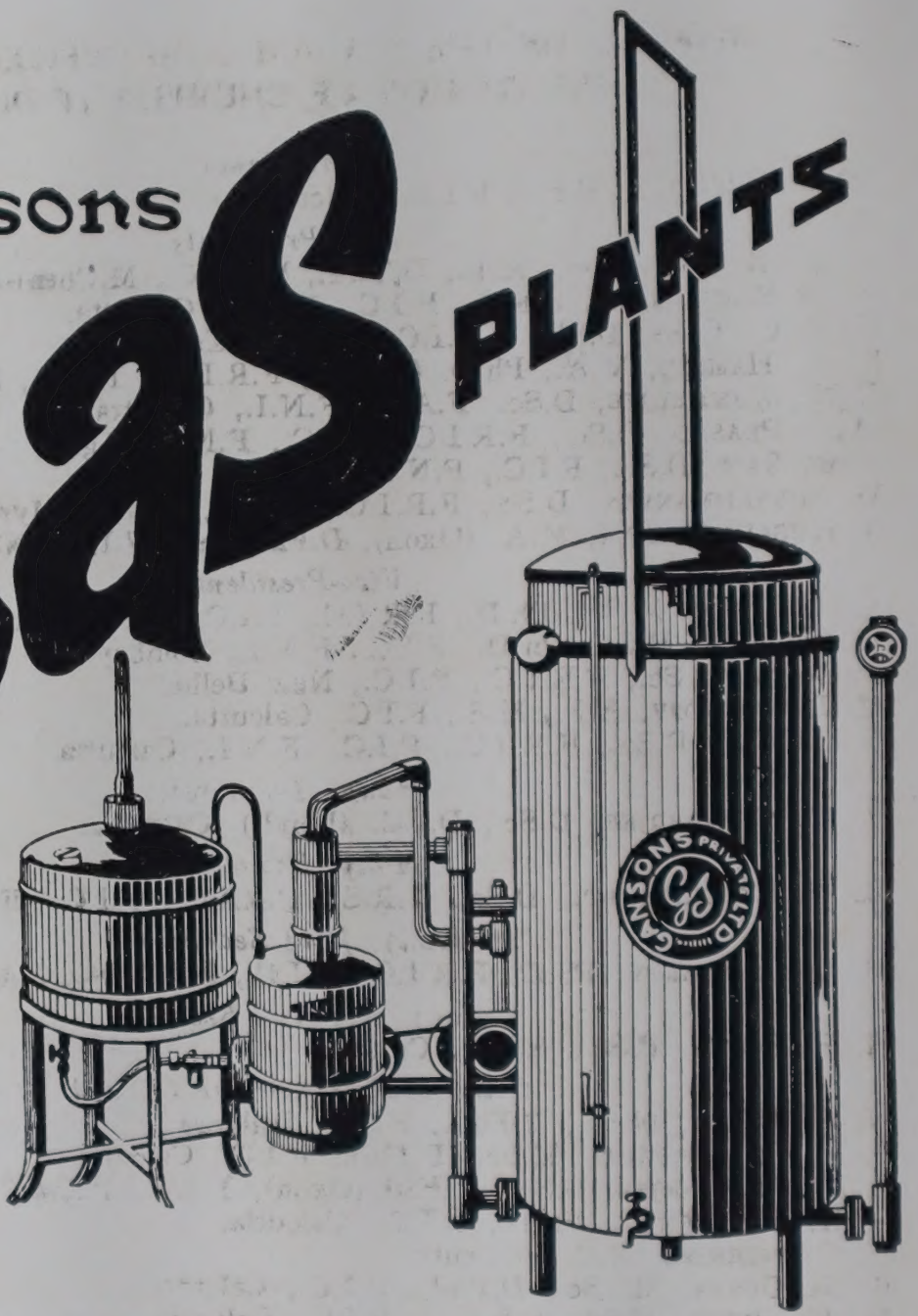

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EXAMINATION FOR THE ASSOCIATESHIP
Group A (Analytical Chemistry)

1. A.I.C. by examination is recognised as equivalent to M.Sc. degree in chemistry for purposes of recruitment of chemists as per G.O. No. F. 18—36/57-T-5, dated 19-8-58 of the Ministry of Scientific Research and Cultural Affairs. The examination with Section 2 (Drugs and Pharmaceuticals) is duly recognised in the Drugs Rules, Government of India. The twentieth Examination is expected to be held at Calcutta in or around November, 1970, and will comprise of Theoretical, Practical and Oral tests.
2. In addition to General Chemistry (compulsory), Group A is divided into the following Sub-groups and Sections. Candidates will be required to select two subjects from amongst these (not more than one from a single Sub-Group):

Sub-Group	*Section*	*Subject*
I	1.	Analysis of Minerals, Silicates, Ores and Alloys.
	3.	Analysis of Foods.
	9.	Analysis connected with Forensic Chemistry.
	10.	Analysis connected with Leather Chemistry.
II	2.	Analysis of Drugs and Pharmaceuticals.
	4.	Analysis of Water and Sewage.
	11.	Analysis connected with Textile Chemistry.
III	5.	Biochemical Analysis.
	6.	Analysis of Oils, Fats and Soaps.
	7.	Fuel and Gas Analysis.
	8.	Analysis of Soils and Fertilisers.

3. Number of theoretical papers is four, each of three hours' duration (Full Marks—100 in each ; Pass Marks—40% of total of theoretical). Paper I contains questions in two parts, one on organic and the other on inorganic chemistry. Paper II also contains questions in two parts, one on physical and the other on general analytical chemistry. Papers III and IV are on the two sections of applied analytical chemistry chosen by a candidate.
4. Each candidate is required to appear at practical examinations in the two sections chosen by him. The practical examination in each section continues for two days—six hours a day (Full Marks—300 in each ; Pass Marks—50% of total of practical).

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6. The examination fee is Rs. 85/-. The last date for submitting the fees is 31st May, 1970. Question papers of previous years are available at Rs. 2/- per year and the Syllabus at Re. 1/- per copy.

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Journal of the
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Vol. XLI MARCH, 1969 Part II

UNIVERSITIES AND TECHNOLOGY

Since independence there has been an accelerated increase in the number and capacities of Universities in the country providing greater and greater scope for training in the pure sciences. As a result it is becoming increasingly necessary to check this serious overproduction of scientists trained in the sophisticated way with consequent increase in unemployment of this category. Following Parkinson's procedure in having more Universities and even morning and evening shifts it may be possible to have a buffer effect within certain limits by employment of more staff and similar measures. Nevertheless, the problem increases in intensity with time. The general feeling is that the country is unable to provide for its trained scientists and so they are staying abroad. Of course, the term 'brain drain' originated in view of conditions in Britain and only its use extended to our country along with many others. It is difficult to stop this if there is a greater demand for trained scientists elsewhere with possibility for greater emoluments. Importation of scientists is like a natural corollary to a country unable to train up the required number of scientists within the land. However, it is important to note that India, inspite of her very limited resources, is training up a band of surplus workers at considerable expense for serving elsewhere.

It is a mass-appealing topic to formulate that University education should be oriented towards the immediate needs of the masses in a convocation address but if the speaker himself be at the helm of University education we should naturally expect to see some positive steps taken. As it stands, more and more scientists are being produced by the Universities, and on the basis of their University training they are becoming fascinated in teaching and sophisticated research instead of looking after the immediate needs of the masses.

Starting of the Institutes of Technologies in the country raised some hope of having a stream of technologists oriented towards the needs of the masses. Unfortunately, instead of concentrating whole-heartedly to this side such Institutes are also trying to indulge in competition with the Universities in the production of 'ivory' tower' scientists adding to the problem of overproduction of such scientists and lowering of the standard. Perhaps these Institutes are not the proper answer. Perhaps there is need for polytechnics.

R. N. C.

J. Inst. Chemists (India), Vol. XLI, March, 1969

GRAVIMETRIC DETERMINATION OF BARIUM FROM HOMOGENEOUS SOLUTION

By

D. P. Goel, K. C. Trikha and R. P. Singh

Department of Chemistry, University of Delhi, Delhi.

(Received for publication on 6-5-68).

The precipitation of barium sulphate for gravimetric determination using the technique of precipitation from homogeneous solution (PFHS) has been reported by a number of workers[1-5]. Heyn and Shupak[4] used the method of cation release and claimed the method to be superior to the sulphamic acid and the dimethyl sulphate[2, 3] methods. The use of sulphite ion has been reported by Vickery[6] in the precipitation of rare earth alkali double sulphates. But doubts have been expressed regarding its categorisation as the PFHS technique as the precipitation takes place immediately upon the introduction of the gas phase.

A new approach to the precipitation of barium sulphate using the sulphite ion as a source reagent for sulphate ions is presented. Barium-ethylenediamine-tetraacetic acid complex is slowly destroyed by hydrogen peroxide, which also oxidises the sulphite ions to sulphate ions, which combine with the released barium cations resulting in a coarsely crystalline precipitate of barium sulphate. The barium sulphate thus formed does not creep up and is readily filterable. This method of precipitation from homogeneous solution involves cation release as well as the generation of the precipitating agent. High rates of oxidation, unlike the precipitation of rare earths alkali sulphates, were not not observed in our case as evidenced by the appearance of first sign of turbidity only after about forty five minutes.

Experimental

Reagents :

Barium solution : Barium solution was prepared from $BaCl_2.2H_2O$ (BDH, AnalaR). The solution was standardised gravimetrically[7].

Other solutions : Reagent grade chemicals were employed in the preparation of other solutions.

Buffer solution (pH=10) : Add 568 ml. of concentrated ammonia solution (Sp. gr. 0·88-0·90) to 70·0 g. of ammonium chloride and dilute to 1 litre with distilled water.

Procedure :

Transfer to a 400 ml. beaker the sample solution containing, 10-120 mg. of barium. Add for every 15 mg. of barium, 2 ml. of 0·1M-EDTA solution (37·2250 g. of $Na_2H_2C_{10}O_8N_2.2H_2O$ per litre), 5 ml. of buffer solution, 5 ml. of 30% hydrogen peroxide and 1 ml. of 10% sodium sulphite solution ($\sqrt{}$100 mg.). Dilute to 250 ml. and keep the solution on a hot plate, when after half an hour first sign of turbidity appears. The digestion is continued for 2 hrs during which period the whole of barium is precipitated. If metals other than barium are present and which also from stable complexes with EDTA, add additional EDTA solution. The pH of the filtrate at the completion of the precipitation is between 7·0 and 8·0. Decant the solution through a filtering crucible. Wash the precipitate with hot water and finally with absolute ethanol and ether. Dry the precipitate in an oven at 110°C and ignite the precipitate at 900°C. The results are calculated using the factor $Ba/BaSO_4$=0·5885. Results of determination of barium alone and in the presence of interfering cations are reproduced in Table I and II respectively.

TABLE I

Estimation of barium as barium sulphate

Amount of barium taken mg	Amount of barium sulphate, mg (Calc.)	Amount of barium sulphate, mg (found)	Amount of barium found, mg	Error in mg
13·74	23·34	23·40	13·77	+0·03
27·47	46·66	46·70	27·48	+0·01
54·95	93·37	93·40	54·97	+0·02
109·89	186·76	186·80	109·95	+0·06
120·00	203·90	204·00	120·05	+0·05

TABLE II

Effect of foreign ions on determination of barium

Amount of Ba taken mg	Foreign ion added	Amount of foreign ion added, mg *	Amount of barium sulphate found, mg	Amount of barium found	Error in mg
13·74	Ca^{+2}	160·00	23·40	13·77	+0·03
	Mg^{+2}	121·60	23·40	13·77	+0·03
	Pb^{+2}	41·44	23·60	13·89	+0·15
	Fe^{+2}	100·82	23·60	13·89	+0·15

* Figures give maximum tolerable units.

Results and Discussions

Barium is complexed with EDTA at high pH values. The barium cation is slowly released from an ammoniacal medium (pH=10) containing the complex of barium with EDTA ; if hydrogen peroxide is added and the solution gently boiled. The oxidising agent destroys the complex and volatilisation of ammonia

results in the dissociation of the barium-EDTA complex. The barium cation thus set free combines with the sulphate ion. The presence of appreciable amounts of ammonium ions improves the crystalline character and larger and uniform crystals are obtained. Similar effect of ammonium ions on the morphology of barium sulphate has been reported by other workers[9, 10].

The procedure has been tested in the presence of calcium, magnesium, lead, iron and strontium. The separation of barium from calcium and magnesium is excellent even when present in considerable excess. Iron and lead in larger amounts interfere. Strontium interferes seriously. Excess of ammonium acetate has been used as a masking agent for lead. With 15 mg. or less of barium present, as much as 40 mg. of lead can be tolerated.

The method compares favourably with the dimethyl sulphate and sulfamic acid procedures so far as the accuracy of results is concerned. In addition, the ready availability and non-toxic character are distinct advantages. This method is superiod to the cation release method employed by Heyn and Shupak[4] in which low results are generally obtained.

Summary

The property of cation release coupled with the oxidation characteristics of hydrogen peroxide has been utilised in the precipitation of barium sulphate from homogeneous solution with sulphite ion as a source reagent. The determination of barium has been carried out in the presence of calcium, magnesium, lead and iron with satisfactory results.

Acknowledgement

The author (D.P.G.) is thankful to the Ministry of Education, Government, of India, for the award of Research Scholarship.

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THE PREPARATION AND REARRANGEMENT OPTICALLY ACTIVE ETHERS WITTING REARRANGEMENT

By

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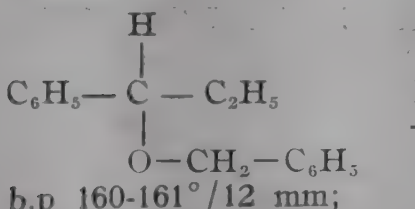
Chemistry Department, Indian Institute of Technology, Hauzkhaus, New Delhi.

(Received for publication on 15-4-68)

In furtherance of study of the rearrangement of optically active benzyl and benzhydryl ethers under the influence of Lewis bases retention of optical activity by the rearranged carbinol has only been observed in ethers, containing phenyl group attached to the potentially active carbon[1]. In the present work (+) and (−) benzyl-1-phenyl propyl ether, b.p. 160-161°—/12mm η^{25}_D 1·5416 ; α^{22}_D (+) 67·5° ; (−) 66·22° (L-1-homo) were prepared in 78-80% yield from (+) and (−) phenylethyl carbinols b.p. 105-8/18mm, η^{22}_D 1·5180; α^{22}_D (+) 29·16; (−) 28·50 (L-1.homo)- respectively, and rearranged by treatment with potassium amide (2 mol) to yield optically active carbinol b.p. 48-50°/0·2 mm; η^{20}_D 1·4900; α^{25}_D +74.50; -74·28°. The rearranged carbinol has been found to be 1, 2 diphenyl-butan-1-01.



b.p 160-161°/12 mm;
α^{22}_D (−) 65·22°; (+) 67·5°

(i) rearranged product 55-60% ; b.p. 48-50°/0·2 mm α^{22}_D (+) 74·50 ; (−) 74·28°
(ii) benzoic acid 8-10%
(iii) residue 25-27% undistilled.

The experiment was repeated using potassium amide and sodium *t* butoxide ; the percentage yields and rotatory powers of the products are tabulated.

Reagent used		Ether taken	Carbinol obtained	Benzoic acid %	Residue %
Potassium amide in ether (2 mols)	I	5·3 g α^{22}_D +67·5°	3·18 g α^{22}_D +74·50°	8·12	28·2
	II	12·2 g α^{22}_D −65·22°	7·58 g α^{22}_D −74·28°	8·3	26·6
	III	10·6 g α^{22}_D +67·5°	6·30 g α^{22}_D +74·46°	8·52	25·5
Sodium-*t* butoxid in t-butanol (2 mol)	I	5·3 g α^{22}_D +67·5°	2·93 g α^{22}_D +74·5°	10·00	27·3
	II	8·4 g. α^{22}_D −65·22°	4·26 g α^{22}_D −74·21°	10·17	26·8

Experimental

Firstly (±) benzyl-1-phenyl-propyl ether b.p. 160-162/12 mm; 119-120°/2mm ; η^{25}_{D} 1·5417 was prepared by interaction of potassium salt of (±) phenyl ethyl carbinol with benzyl chloride. (±) Benzyl-1-phenyl propyl ether was treated under reflux for 10 hours with potassium amide (2 mol) in diethyl ether, sodium *tert*-boutoxide (2 mol in *tert*-butyl *alcohol* and phenyl lithium in diethyl ether separately. Phenyl lithium did not bring about any rearrangement of benzyl-1-phenyl-propyl ether and 91% of the unchanged ether was recovered. The dark-brown solutions-suspensions resulted on treatment with potassium amide and sodium text butoxide on working up yielded colourless viscous liquid b.p. 118-120°/2 mm ; η^{25}_{D} 1·5146. Potassium amide and sodium-tert-butoxide were found to be suitable reagents for rearrangements.

Resolution of (±) Phenyl — ethyl cardinol.

(±) Phenyl-ethyl carbinol b.p. 105-108°/18 mm η^{20}_{D} 1·5200 was resolved into its optically active antipodes by preparing its (±) hydrogen phthalate in presence of pyridine. (±) hydrogen phthalate m.p, 164·5°—recrystalised from ether-petroleum ether-was obtained in 74% yield. The (±) hydrogen phthalate was resolved into (+) and (−) hydrogen phthalates m.p. 160-161°; α^{CS2}_{D} (+) 91·54; 69·68% and m.p. 149·50° α^{CS2}_{D} (−) 89.20; 50% respectively by formation of their quinidine salts and subsequent removal of quinidine by treatment with HCl (1 : 1). The (+) and (−) Phenyl-ethyl carbinol b.p. 105-8°/18 mm;. η^{25}_{D} 1·5180; α^{22}_{D} (+) 29·16; (−) 28·50° (1.1.homo) in 75-77% yields, were liberated from the optically active hydrogen phthalates by hydrolysis with NaOH solution (6 vols).

Potassium salt of (−) phenyl-ethyl cardinol.

Potassium (3·9 g; 0·1 mol) was melted under stirred xylene (50 ml) and allowed to cool. To the fine suspension (+) phenyl-ethyl carbinol (13·6g ; 0·1 mol) was added dropwise during one hour, and continued stirring for further two hours. The mixture was then heated under reflux until reaction was complete.

Benzyl (+) -1-phenyl-propyl ether.

To the warmed and stirred fine suspension of potassium salt of (+) phenyl-ethyl carbinol, benzyl chloride (12·6 g; 0·1 mol) in xylene (20 ml) was added during two hours with stirring. After stirring for another hour filtered off sodium chloride and washed the filtrate with water, dried and distilled. (+) Benzyl-1-phenyl-propyl ether b.p. 160-161°/12 mm. η^{25}_{D} 1·5416 was obtained in 80% yield. It redistilled at 119-120°/2 mm α^{25}_{D} (+) 67·5 (homo) : Similarly (+) Benzyl-1-phenyl propyl ether b.p. 118-120°/2 mm; η^{25}_{D} 1·5417, α^{22}_{D} (−) 65·22 (1.1.homo) was obtained in 90% yield.

REARRANGEMENT OF OPTICALLY ACTIVE BENZYL-1-PHENYL-PROPYL ETHER

(a) *By the action of Potassium amide in liquid ammonia.*

(+) Benzyl-l-phenyl-propyl ether $\alpha^{\cdot}_{D}$ (+) 67·50° (0·1 mol) in anhydrous diethyl either was stirred with potassium amide (0·2 mol) potassium metal dissolved in liquid ammonia (200 ml) for 80 hours. The resulting brown mass was then decomposed with water (50 ml) and shaken with water (500 ml) and ether (500 ml). The ethereal layer after drying over anhydrous sodium sulphate and freeing from the solvent yielded :—

Fraction I b.p. 48-50°/0·2 mm η^{25}_{D} 1·4900; 60% α^{25}_{D} (+) 74·50°

Fraction II b.p. 118-120/0·2 mm $^{25}_{D}$ 1·5416 30% α^{CS2}_{D} +38·00°—unchanged ether.

Residue *did not distil*

Similarly (−) Benzyl-l-phenyl—propyl ether $\alpha^{\cdot}_{D}$ (−) 65·25° on rearrangement with potassium amide yielded :—

Fraction I b.p. 48-50°/0·2 mm; η^{25}_{D} 1·4900; 58% $\alpha^{\cdot}_{D}$ (−) 74·28°

Fraction II b.p. 118-120°/0·2 mm. η^{25}_{D} 1·5416; 33·5% α^{CS}_{D} 2(−) 36.80—unchanged ether

Residue—did not distil.

Fraction II—on keeping deposited benzoic acid.

(b) *By the action of sodium—tert—butoxide.*

A mixture of (+) benzyl-l-phenyl ether (0·1 mol) and sodium-tert-butoxide (0·2 mol) in tertiary butyl alcohol (150 ml) was refluxed on water bath for 10 hour—total contact time 30 hours. The deep red solution after the usual treatment yielded :—

Fraction I b.p. 48-50°/0.2 mm.— $\eta^{\cdot}_{D}$ 1.4900; 55% $\alpha^{\cdot}_{D}$ +74.52°

Fraction II b.p. 118-120°/0·2 mm.—unchanged 35·8% ether

Residue — did not distil

Characteristisation of the rearranged product.

The optically active compound obtained on rearrangement of the optically active benzyl-l-phenyl-propyl ether has been found to be 1, 2 diphenyl-butan-1-01 by formation of its acetyl and urethane derivatives.

(i) *Acetyl derivative of the rearranged product.*

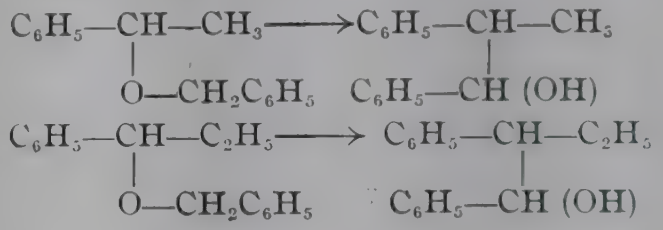
A vigorous reaction started on adding acetyl chloride (0·7 g) to the (+) rearranged carbinol (1g). After five minutes when the reaction stopped, poured the solution onto ice-water (15 ml) and extracted the viscous liquid liberated with ether. The ethereal layer after washing with water, sodium carbonate solution and again with water was dried over sodium sulphate. On removing the last traces of the solvent by passing air through it. A white crystalline residue m.p. 103-105° was obtained after crystallisation from ether and light petroleum ether; it had m.p. 107° 0·9; mixed melting point with authentic sample was 106-107°; α^{22}_{D} (+) 18·6° (1, 1 alc).

Urethane derivative of the rearranged product.

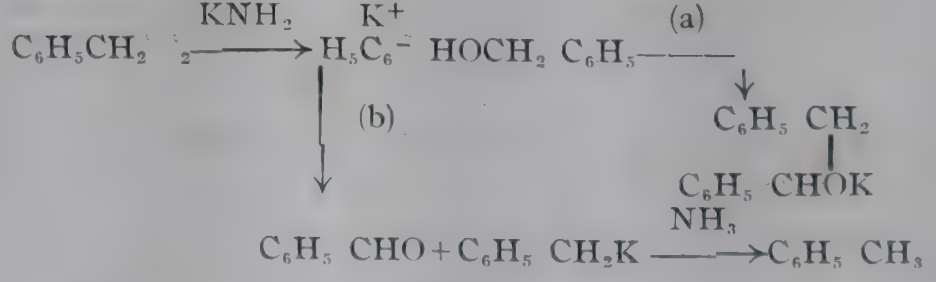
To a solution of (+) rearranged product (2·0g) in light petroleum ether added an equal volume of phenyl isocyanate and heated the solution at 40-45° for one hour. Recrystallisation of the resulting white substance from petr. ether gave white crystalline urethane m.p. 115-116°; mixed melting point with authentic sample 115-116°;

Conclusion

The rearrangement of optically active benzyl-l-phenyl-ethyl ether and benzyl-l-phenyl-propyl ethers to the corresponding optically active carbinols establishes the intramolecular nature of the reaction.



The presence of benzoic acid in the rearranged carbinols indicates that the rearrangement of benzyl and related ethers is always accompanied by a side reaction of the intermediate resulting in the isolation of benzaldehyde (obtained as benzoic acid) and toluene. The formation of the latter substance is shown by Hauser in course (b) in the equation.



It confirms that the rearrangement is not followed by B-elimination because the rearranged carbinol does not undergo any change on treatment with potassium amide under similar experimental conditions. Thus, the formation of B-elimination products and stability of rearranged carbinols on treatment with potassium amide shows that the rearrangement of ethers takes place in two steps (i) metallation (ii) rearrangement of the metallated ether.

ACKNOWLEDGEMENT

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A PAPER CHROMATOGRAPHIC METHOD OF DETECTION AND ESTIMATION OF INDIVIDUAL SUGARS PRESENT IN FOOD STUFF IN PRESENCE OF ONE ANOTHER—MILK

By

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The problem of detection and estimation of small amount of added sugar in milk which was not very confirmative by classical tests, specially in presence of formalin (as a preservative) which interfers with the resorcinol test[1], let us to try some simpler paper chromatographic method.

Ever since Patridge[2] used paper partion chromatography for the separation and detection of sugars, many workers have reported their quantitative estimation by (1) extraction and determination by suitable micro method, (2) staining and reading the coloured spots by direct photometry, and (3) elution followed by photo-colorimetric estimation.

Most of the methods were tried and numerous chromatographs were run under different conditions. The following method was found best suitable for routine analysis of sugars in milk. Some of the results are given in Table I.

Experimental

(a) Treatment of the milk sample—

About 5 cc of the milk is taken in a test tube, 0·5 g. of lead-acetate added, and the milk boiled for half a minute. Then about 1 g. of potassium oxalate is added and the test tube is thoroughly shaken. The coagulated milk is filtered. The filtrate is used for chromatography along with standard sugar solutions. Sometimes, when the milk contains too much fat, as in Buffalo milk, the latter is to be removed by shaking repeatedly the above filtrate with ethanol.

(b) Chromatography—

(i) The Chromatography is done in the descending order.

(ii) The *paper* used is whatman No. I filter paper of 50 cm length.

(iii) *Time* taken—18 hours.

(iv) *Solvents* :—Among the numerous solvents employed the following are best suitable for separation of sucrose and milk sugar (lactose).

1. n-butanol/Acetic acid/H_2O (4 : 4 : 1)
2. t-butanol/Acetic acid/H_2O (4 : 4 : 1)
3. n-butanol/Acetic acid/H_2O (4 : 1 : 1)

(v) *Spraying reagent*[3] : Pthalic acid (0·93 g.) and distilled aniline (1·60 g.) mixed together in 100 cc water saturated n-butanol.

Solvent : t-butanol/Acetic acid acid/H_2O (4 : 4 : 1)

Result and Discussion

TABLE I

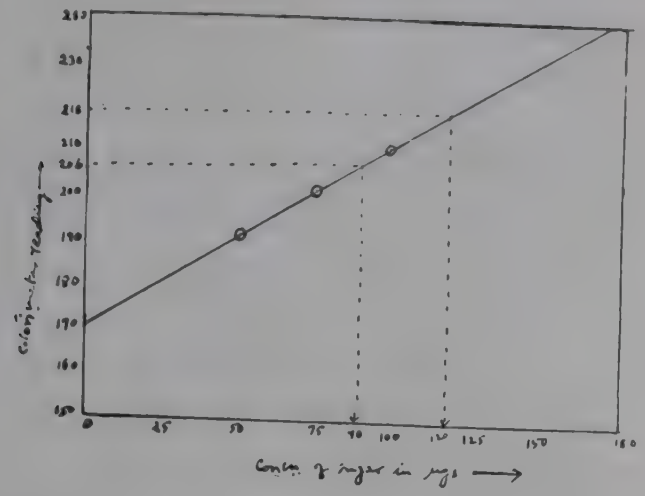
No.	RF of the diff. spots						Remark
	Lactose	Sucrose	Glucose	Fructose	Sample	Formalin	
1.	0·14	—	—	—	0·14	NO SPOT	Sugar not added
2.	0·14	0·32	—	—	0·14 0·32		Sucrose present. Sugar added
3.	0·14	—	0·33	0·40	0·14 0·33 0·40		Glucose and Fructose present Sugar added and hydrolysed

(c) *Estimation*

The following photo-colorimetric method, as suggested by Misra & Rao[4] is adopted with slight modification.

Chromatograph of samples (Milk) are run in the usual way along with standard sucrose solution, sprayed with aniline hydrogen pathlate and heated at 150°C for 5 minutes. Strips of equal area (covering the entire spot) are cut from the sucrose spots of the sample along with those from the standard ones. Colour from the strips are eluted by 55-60% acetic acid (or 100% methanol) by dissolving them in the solvent for 30 minutes and shaking intermittently. The intensities of the colours of the sample solutions are compared with those of the standard ones in a klett-summerson colorimeter using blue filter 450-500 *mu*. The relation between the intensity of colour and corresponding sugar concentration being a rectilinear one, a straight line is obtained by plotting standard concentrations against corresponding intensities in a graph. From the graph, the sugar concentration of the sample solution is found out. Some of the results are given below :—

Concen. of sugar	colorimetric reading
50 *µg.*	190
75 *µg.*	200
100 *µg.*	210
S_1 (6 *µl*)	206
S_2 (6 *µl*)	218



From the graph,
S_1 = 90 µgs
S_2 = 120 µgs

Concen. of sucrose = 1.5%
—Do— = 2%

(About 1·5% sucrose was added to the 1st and 2% to the 2nd Sample).

The Table II below shows the actual amount of sugars added in milk samples and the corresponding amounts recovered :

TABLE II

No. of sample	Amount of sucrose added	Amount recovered by analysis	
	Per µl of milk	Per µl	Recovery %
1.	10 µg.	10 µg.	100
2.	15 µg.	15 µg.	100
3.	20 µg.	18 µg.	90
4.	25 µg.	24 µg.	96
5.	30 µg.	28 µg.	93
6.	35 µg.	33 µg.	94
7.	40 µg.	40 µg.	100
8.	45 µg.	44 µg.	98
9.	50 µg.	48 µg.	96

This method is easy and simple and can be applied to estimate sucrose, when other non-reducing sugars are also present. It is possible to detect even 0·10% of sucrose and estimate upto 1% of the same by this method.

In each operation (requiring one working day) at least 12 samples can be analysed.

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STUDIES ON VITAMIN A COMPLEXES : PART II. INFLUENCE OF BILE ACIDS, WAX AND OTHER MATERIALS*

By

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(Received for publication on 1-2-68).

The importance of bile for the absorption of preformed vitamin A has been demonstrated by Bernhard *et al*[1] and they have claimed that the bile pigments act as antioxidant for the vitamin. Diversion of the bile reduced the absorption to only 1%. An improvement in absorption could be secured by giving natural bile. Administration of taurocholic acid, sodium cholate or sodium taurocholate also showed some improvement. *In vitro* studies have shown that the bile pigments protected vitamin A from oxidation, and also retarded the formation of peroxides by linoleic acid. An interesting observation has been reported by Schlenk *et al*[2], and later by Wakely[3] that vitamin A palmitate forms an adduct with deoxycholic acid. The crystalline adduct so obtained has shown marked superiority to commercial mixtures of vitamin A in respect of both stability and utilization. Another method to increase the stability of vitamin A in foods may be to coat the vitamin with some other material. Thus, superior stabilization of vitamin A in a dry carrier preparation on using spermaceti, esparto wax or microcrystalline wax has been reported in a few patents[4-6]. In view of the above it was considered worthwhile to study the influence of bile acids and their derivatives and of wax on the keeping property of vitamin A.

EXPERIMENTAL

Analytical methods : The method of determination of vitamin A was the same as described in the previous paper[7].

Preparation of the complexes : Vitamin A palmitate (1·7 m. I.U. per g.) has been used throughout this work. The final products having the potency of 20,000-30,000 I.U. per g. are termed as low and those with potency of about 60,000 I.U. or more as the high level of vitamin A.

(a) *As adduct of deoxycholic acid* : The adduct of deoxycholic acid with vitamin A palmitate was prepared at the high level by the method of Schlenk *et al*[2] with some modification. Vitamin A palmitate (0·3 g), α-tocopherol acetate (0·09 g.) and lecithin (0·15 g.) were dissolved in 15 ml. of dehydrated

* Based, in part, on the Thesis submitted for Research Diploma of the Royal Institute of Chemistry, London.

alcohol. This solution was added to a solution of doxycholic acid (3·0 g.) in 40 ml. dehydrated alcohol. The mixture was concentrated on a water-bath at 55° under suction. When the volume was reduced to about 7 ml., the mixture was allowed to cool and kept in the refrigerator for 20 hours. The residue obtained was filtered, washed twice with 2 ml. portions of dehydrated alcohol, allowed to dry in the air for 6 hours and then overnight in a vacuum desiccator.

(b) *As dried preparation with bile acids* : Vitamin A palmitate (0·075 g.) α-tocopherol acetate (0·012 g.) and lecithin (0·012 g.) were dissolved in 15 ml. dehydrated alcohol. This solution was added to a solution of 3 g. of a bile acid (cholic, dehydrocholic or deoxycholic) in 40 ml. dehydrated alcohol. The mixture was warmed for 15 minutes at 55° and the whole mass was poured in a flat dish and allowed to evaporate in the air overnight. The residue was dried in a vacuum desiccator for 48 hours.

(c) *As dried preparation with cholesterol* : The method of preparation was similar to that with bile acids with the alteration that the solvent was chloroform instead of dehydrated alcohol since cholesterol is much less soluble in alcohol.

(d) *With wax or cetyl alcohol* : These samples were prepared by dissolving within five minutes appropriate quantities of vitamin A palmitate (0·075 or 0·3 g.), α-tocopherol acetate (0·012 or 0·09 g.) and lecithin (0.012 or 0.15 g.) in molten wax or cetyl alcohol at about 67°. The mixture was cooled and the solid was taken out by scraping. Three grammes each of paraffin wax, lanette wax and cetyl alcohol were used for each preparation.

(e) *With sodium salt of bile acids* : These complexes were prepared only at the low level of vitamin A. Three grammes each of cholic, deoxycholic and dehydrocholic acids were suspended in water (45 ml.), neutralised with a solution of sodium hydroxide (6%) to pH 7·0 and filtered. Vitamin A palmitate (0.075 g,), α-tocopherol acetate (0·012 g.) and lecithin (0.012 g.) were dissolved in Tween 60 (polyoxyethylene sorbitan monostearate : 0·6 g.) at 55°. To this mixture, was added the previously prepared solution of sodium salt of bile acids and the whole mass was evaporated to dryness at 50° *in vacuo*.

The vitamin A compositions as mentioned above were stored in well closed glass vials in a refrigerator (2°-5°).

Results and Discussion

Vitamin A contents of the compositions were determined at intervals of six months and the results are shown below.

1. *With bile acids, their salts and cholesterol* :

(i) *Bile acids* : In this series three acids namely cholic, dehydrocholic and deoxycholc acids were used. In order to bring the experiments in the line with others as described previously[7], all the adducts were not separated.

Instead, the vitamin A adduct along with the unreacted materials were evaporated to dryness and the results are shown in Fig. 1. The deoxycholic acid adduct was prepared and studied for comparison.

The good stability of vitamin A—deoxycholic acid adduct may be noted from the Figure. The composite product without separation of the adduct had much inferior stability to the corresponding isolated product. But among the three acids studied deoxycholic acid was found to be the least harmful. Dehydrocholic acid was the most deleterious while cholic acid stood in between the other two.

(ii) *Sodium salts of bile acids* : Since the bile acids were destructive to vitamin A it was considered worthwhile to study the effect of sodium salts of cholic, dehydrocolic and deoxycholic acids on the autoxidation of vitamin A. As the sodium salts were water soluble, vitamin A had to be dispersed with the aid of Tween 60. Results are shown in Fig. 2.

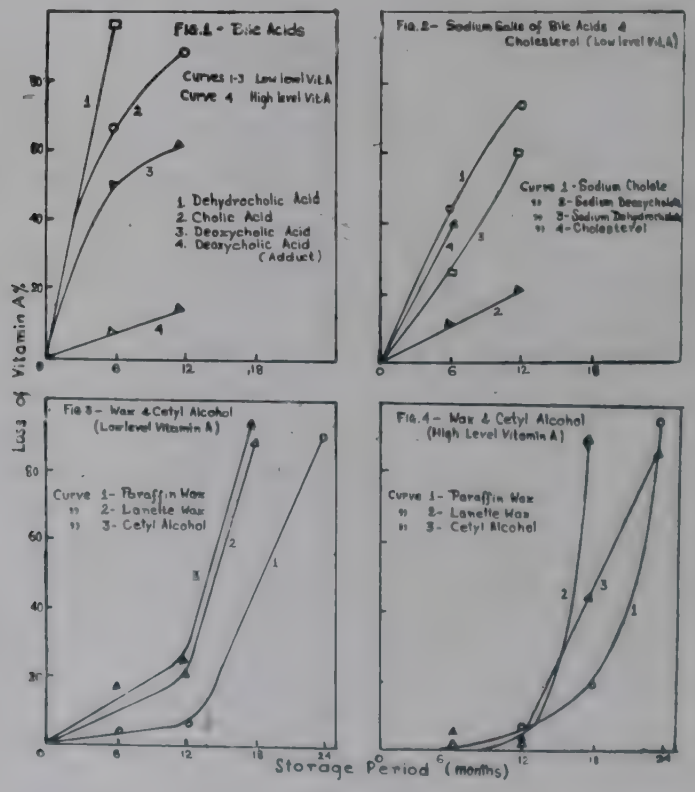
Fig.1 - Bile Acids
Curves 1-3 Low level Vit.A
Curve 4 High level Vit.A
1 Dehydrocholic Acid
2 Cholic Acid
3. Deoxycholic Acid
4. Deoxycholic Acid (Adduct)
Fig.2- Sodium Salts of Bile Acids & Cholesterol (Low level Vit.A)
Curve 1 - Sodium Cholate
Curve 4 - Cholesterol
Fig.3 - Wax & Cetyl Alcohol (Low level Vitamin A)
Curve 1- Paraffin Wax
2- Lanette Wax
3- Cetyl Alcohol
Fig.4 - Wax & Cetyl Alcohol (High Level Vitamin A)
Curve 1- Paraffin Wax
2- Lanette Wax
3- Cetyl Alcohol
Loss of Vitamin A%
Storage Period (months)

Sodium salts of the above acids were much less destructive than the corresponding acids. Sodium deoxycholate exerted significant resistance to autoxidation of vitamin A. Another feature of the study is that sodium dehydrocholate showed slightly better protection than sodium cholate, although the position was just the reverse with the corresponding acids (Figs. 1 and 2).

(iii) *Cholesterol* : The influence of cholesterol on the stability of vitamin A is shown along with those of sodium salts of bile acids in Fig. 2 From the data it is quite evident that cholesterol accelerated the rate of oxidation of vitamin A.

2. *With wax and cetyl alcohol* : Results obtained with samples containing paraffin wax, lanette wax or cetyl alcohol at low level of vitamin A are shown in Fig. 3 and those at the high level are presented in Fig. 4.

At both the levels of vitamin A paraffin wax resisted the autoxidation of vitamin A remarkably well upto 12 months and then the destruction started and it became almost complete in 24 months. The behaviour of lanette wax and cetyl alcohol at low level of vitamin A was almost like paraffin wax in pattern, the only difference being that the extent of destruction of vitamin A in these two cases was greater at 12 months and that it became almost complete by 18 months. At higher level cetyl alcohol and lanette wax behaved upto 12 months exactly like paraffin wax, and thereafter lanette wax caused quicker destruction. At this stage (18 month's storage) cetyl alcohol was less destructive than lanette wax and paraln wax was still less.

Good stability of vitamin A was obtained (Fig. 1) with the vitamin A—deoxycholic acid adduct[2, 3]. The inferior stability of the product containing deoxycholic acid, prepared without separation of the adduct, may be due to the impure adduct preparation which autoxidises rapidly, although the host molecules are present in large amounts[2]. Besides this, deoxycholic acid and the other bile acids in this case appear to behave like fatty acids and help autoxidation of vitamin A. That the acidic nature of the bile acids is responsible for the rapid destruction of vitamin A finds some support from the observation that the corresponding sodium salts are much less deleterious (Figs. 1 & 2). Sodium deoxycholate appears to exert a protective action. The influence of cholesterol on the stability of vitamin A received our attention since there have been reports of some relationship between vitamin A and cholesterol *in viro*[8, 9]. The relative decreased stability of vitamin A in presence of cholesterol (Fig. 2) may be one of the possibele reasons why a reduction in the storage of vitamin A in the liver was observed by other workers[10, 11] when cholesterol was given together with the vitamin. The above observation of inferior stability of vitamin A agrees with the reported interference[11] between vitamin A and cholesterol since in humans with increased cholesterol content in the blood, a reduction has been found to result from ingestion of large quantities of vitamin A[12] and high doses of vitamin A reduces cholesterolemia induced in chicks by feeding cholesterol[13].

The good stability of refrigerated vitamin A preparations containing paraffin wax, lanette wax and cetyl alcohol upto storage for 12 months (Figs. 3 & 4) and the subsequent sharp destruction[14, 15] indicate an initial induction period. In the accelerate studies (at 98°) the result of Dann[15] also indicated an induction period (about 4 hours). The wax or cetyl alcohol seems to form a protective coating on vitamin A and thus appears to prevent the immediate contact with oxygen with the consequent increase in the induction period.

Summary

Bile acids (cholic, dehydrocholic and deoxycholic acids) and cholesterol in general helped autoxidation of vitamin A, the deoxycholic acid being less

deleterious than other bile acids. Good stability was obtained with vitamin A—deoxycholic acid adduct. The sodium salts of bile acids on the other hand were much less destructive than the corresponding acids, and sodium deoxycholate actually appeared to exert a protective action. The wax (paraffin and lanette) or cetyl alcohol seems to form a protective coating on vitamin A and thus appears to prevent the immediate contact with oxygen with the consequent increase in the induction period.

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LOW VACUUM DIFFUSION-OXIDATION MICRO TEST FOR BLOOD ALCOHOL

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In author's previous work[1], the principle of indirect elimination of error from volatile interfering factors present in laboratory air was based on a presumption (without verification of its correctness) : equal volumes of laboratory air and therefore, equal quanta of vitiating influences from this extrinsic source were drawn in through the two identical wings of apparatus, one for test blood and the other for control sample, subjected to identical physical conditions of aeration experiment. It was on the basis of this presumption that error from laboratory air was eliminated as a common factor by calculating the difference between amounts of dichromate reduced in test and control experiments to arrive at the correct value for true alcohol content of test blood.

In the present vacuum diffusion micro method, reported in this communication, no presumption was made in processing the technic. Another sharp contrasting feature is that pure air-current was harnessed through the agency of apparatus, Fig. 1 to create vacuum only, as against the practice followed in aeration technic, in which air is bubbled through the test blood.

Experimental

Apparatus :

The design and contruction of apparatus in its two wings and orientation, function, etc of its components are fully explained in Fig. 1.

Reagents :

1. Scott-Wilson reagent[2] for removing normal volatile metabolites present in test blood; 2 Potassium dichromate, 0.1 N; 3. Sodium thiosulphate, 0.05 N and 0.01 N, to fill in two 2-ml micro burettes, each capable of being read to 0·01 ml; 4. Potassium iodide crystals, reagent grade; 5. Concentrated sulphuric acid, reagent grade; 6. 1% Starch solution freshly made every day.

Procedure :

Test blood : residual blood was selected in the present work. Varying known quantities of standard alcohol (vide 'infra') were added to measured

volumes of test blood and experimentally recovered. Standard alcohol : two standards, one with 1 in 500 dilution and the other with 1 in 2000 dilution were prepared from absolute alcohol .

1 ml of each was directly oxidised with 0·1 N potassium dichromate concentrated sulphuric acid mixture (each of 10 ml) and their alcohol values, iodometrically determined, were respectively 0.9773 mg and 0.2443 mg.

Into the wash-trap (flask A, Fig. 1) and the reaction flasks (C, C) were measured out the reagents (RM) as noted in Fig. 1 0.10 ml Scott-Wilson reagent was pipetted into the test glass cup (B), followed by 0.1 ml test blood run from Ostwald-Folin pipette, rinsed out with 0·1 ml of glass-distilled water that was added to the test blood in cup (B), rolled between palms to mix contents (BRt, Fig. 1). A known quantity of alcohol was then added and mixed. Into the control cup (B) was pipetted 0.10 ml Scott-Wilson reagent alone (Rt, Fig. 1). The cups (B, B) were then connected as shown in Fig. 1, the aspirator set to work and the air-current continued for 15 minutes at temperature of boiling water-bath. During that interval the contents of cups dried up. A series of experiments was repeated with known quantities of standard alcohol added to test blood. The calculated difference in volume of 0.1 N dichromate reduced in test and control reaction flask (C, C), Fig. 1, determined iodemetrically, represented the equivalence of true alcohol thus recovered (vide Table 1).

Results and Discussions

TABLE I

Results of alcohol recovery

Experiment	Volume of test blood in ml	Original alcohol content in mg	Added alcohol in mg	Total alcohol content in mg	Alcohol recovery* in mg	Percent increase(+) or decrease(−) in recovery figure
1	0·10	0·0305	0·0517	0·0822	0·0819	−0·36
2	0·10	0·0305	0·1035	0·1340	0·1347	+0·52
3	0·10	0·0305	0·1552	0·1857	0·1864	+0·48
4	0·10	0·0305	0·2070	0·2375	0·2364	−0·46
5	0·10	0·0305	0·2587	0·2892	0·2881	−0·34
6	0·10	0·0305	0·3204	0·3509	0·3494	−0·42
7	0·10	0·0305	0·4239	0·4544	0·4556	+0·26
8	0·10	0·0305	0·4756	0·5061	0·5085	+0·47

* Figure represented mean of duplicate test.

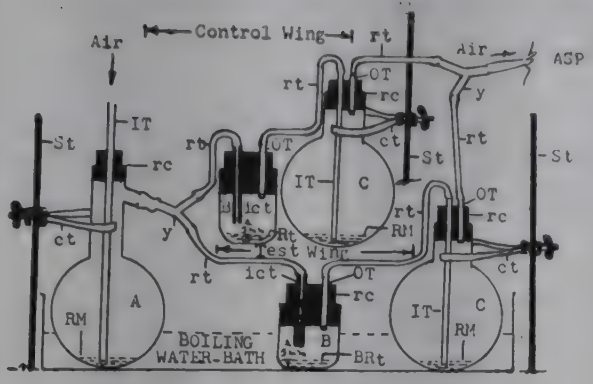

FIG. 1

A : 250-ml round bottom pyrex glass flask with side tube containing 1 ml 0·1 N $K_2Cr_2O_7$ + 4 ml conc. H_2SO4 (RM) to function as wash-trap,
B, B : Cylindrical pyrex glass cups, 30×50 mm, containing 0·10 ml test blood + 0.10 ml Scott-Wilson reagent (BRt) in test wing and 0·10 ml Scott-Wilson reagent (Rt) in control wing,
C, C : 250-ml round bottom pyrex glass flasks with short neck, 4·00 cm, each containing 1 ml 0·1 N $K_2Cr_2O_7$ + 4 ml conc. H_2SO4 (RM) in the test and control wings respectively,
rc : Rubber cork,
rt : Rubber tube,
IT : Inlet glass tube,
ict : Inlet capillary glass tube in cups (B, B),
OT : Outlet glass tube,
St : Metal stand provided with catch (ct) for holding neck (and flask) vertically,
ASP : Aspirator pump,
y, y : Y-glass tubes, one connected with side-tube of wash-trap (flask A) and the other with aspirator pump (ASP).

TABLE II

*Reduction of dichromate*** in wash-trap (flask A, fig, 1)*

I Experiment ...	1	2	3	4	5	6	7	8
II 0·1 N $K_2Cr_2O_7$ reduced ...	0·0020	0·0030	0·0020	0·0010	0·0020	0·0020	0·0010	0·0030
III Equivalent alcohol in mg (figure in Col. II multiplied by 1·15) ...	0·0023	0·0034	0·0023	0·0011	0·0023	0·0023	0·0011	0·0034

*** Quantum of error likely to be included from vitiating factors present in laboratory air would, obviously, be dependent on the technic employed (diffusion or aeration or distillation) and duration of experiment.

Fluctuation in the figure of reduction of dichromate is a pointer to changing condition of laboratory atmosphere during the day's business.

Previous methods have not prevented error from either of the dual source of vitiating factors, intrinsic and extrinsic. The special distillation technic of Kozelka[3, 4] and Hine, and the diffusion method of Cavett[5] have eliminated error from intrinsic volatile metabolites normally present in test blood, but the extrinsic interfering factors likely to be present in laboratory air and in reagents[6] were not screened in these method; McNally[7] and Coleman bubbled air through concentrated sulphuric acid to first purify and then employ it in their aeration technic. But this acid is not an ideal purifier of air : gases of the paraffin series (alkanes, alkenes etc), for example, are not acted upon by

this acid alone. Above all, these workers have not prevented error from intrinsic normal volatile metabolites.

The apparatus (Fig. 1), designed on original lines, has performed the role of key-weapon in efficiently fighting out the vitiating factors in the following way: (i) fractionation of test blood alcohol from normal metabolites by treatment of the specimen with Scott-Wilson reagent which prevented error from *intrinsic* source; (ii) error from *extrinsic* sources (laboratory air and reagents) was prevented in two instalments—(a) vicious factors present in laboratory air were *directly* eliminated, en masse, in the washtrap (flask A, fig. 1) and (b) volatile factors present, if any, in reagents were *indirectly* prevented by control test. These processes, thus integrated, have therefore left no room whatsoever for presuming things in arriving at accuracy in test results at 100 percent $\pm 0{\cdot}4$ (average of 8 experiments, Table 1).

Kinetics involved in the present technic were regulated as follows: (i) disparity in bore of inlet capillary tubes (ict, 0·2 mm) and outlet tubes (OT, 4·0 mm) helped creation of vacuum by air-current in cups (B, B), fig. 1; (ii) vacuum thus created encouraged rapid diffusion-separation of alcohol (and volatile substances present in reagents); (iii) high vapour pressure of alcohol (about 10 times of aqua) also helped its rapid release; (iv) distillation in vacuum (i, ii) was also an accompaniment; (v) temperature of boiling water-both generally accelerated these processes (i, ii, iii, iv); (vi) the air-current served as carrier of cargo of diffused substances for the latter's discharge into and oxidation by corresponding standard acid dichromate set up in reaction flasks (C, C), Fig. 1.

Conclusion

The approach thus made in the present work to solve the original dual problems of prevention of error from intrinsic and extrinsic sources, has yielded reliable and accurate results. The extrinsic vicious factors present in laboratory air were directly eliminated, *en masse*, in wash-trap (flask A, Fig. 1).

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EFFECT OF MANURE AND FERTILIZER ON THE ORGANIC CARBON RESERVE IN THE ACIDIC RED LOAM SOIL OF CHOTANAGPUR

By

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There is no denying the fact that the tropical soils are generally low in organic carbon as compared to the soils of temperate regions. The sedentary red-loam upland soils of Chotanagpur (Bihar) have a low level of fertility. They are low in organic carbon (0·5-0·6%), acidic in reaction (pH 5·4-5·6) and poor in total nitrogen. Experiments carried out during the last 10 years have indicated that improvement in the fertility of the soils of the region can be brought about by addition of lime and fertilizers (Mandal *et al.*, 1966,[1, 2] Rai *et al.*, 1963[3]). Even then, the productivity level of these soils seems to be lower than that of the Gangetic alluvium of the State. This may be due to low organic carbon contents in these soils. Jenny and Raychaudhuri (1960)[5] have established the fact that climate and cultivation have got a great effect on the nitrogen and organic matter reserve of Indian soils. In order to raise the fertility status of the upland soils of Chotanagpur in Bihar, a field experiment was started during 1956-57 at the Agricultural Research Institute, Kanke, in a permanent experimental plot. Since then, various treatments including the use of bulky organic manure (F.Y.M.), fertilizers and mixture of the two in various proportions have been made to study their effects on the soil fertility and crop yields.

Experimental

The field experiment was laid out with the following treatments :

1. Control
2. N @ 40 lbs/acre as A/S (N)
3. N @ 40 lbs/acre as F.Y.M. (F.Y.M.)
4. N @ 20 lbs/acre as A/S+N @ 20 lbs/acre as F.Y.M.+ $x/2P_2O_5$ as S.S.P. $+y/2$ K_2O as M.P. ($N\frac{1}{2}$+F.Y.M.$\frac{1}{2}$+P$x/2$K$y/2$)
5. N+P_2O_5 @ 40 lbs/acre as S.S.P. (NP)
6. F.Y.M.+(40−x) P_2O_5 as S.S.P., F.Y.M.+P (40−x)
7. F.Y.M.$\frac{1}{2}$+N$\frac{1}{2}$+P(40−x/2)
8. N+P+K_2O @ 40 lbs/acre as M. P. (NPK)
9. F.Y.M.+P(40−x)+(40−y) K_2O as M.P. (F.Y.M.+P (40—x) K (40—y)
10. F.Y.M.$\frac{1}{2}$+N$\frac{1}{2}$+P(40−x/2) K(40−y/2)
11. L (lime)+N.P.K.

12. L+F.Y.M.+P (40—x) K (40—y)

A/S	—	Ammonium sulphate
S.S.P.	—	Single super phosphate
M.P.	—	Muriate of potash
x	—	Amount of P_2O_5 in F.Y.M.
y	—	Amount of K_2O in F.Y.M.

The treatments were replicated four times. Lime was added according to lime requirement of the soils once in 4 to 5 years. Organic carbon was determined according to the procedure of Walkley and Black as given by Jackson (1958)[1]. Manures (F.Y.M.) and fertilizers were added twice in a year before the sowing of maize in kharif and of wheat in rabi seasons year after year, i.e., maize-wheat rotation was followed.

Results and Discussion

Physicochemical & chemical data in respect of the soil sample before the start of the experiment, yearwise analysis of the soil samples (organic carbon expressed as mg per 100 gms of soil) and mean yields of maize and wheat (10 years' mean) are presented in Tables 1, 2 and 3 respectively.

TABLE I

Analysis of the soil sample before the start of the experiment

pH	Total N%	Organic C%	Exchangeable bases (m.e. %)			Available P in soil ppm
			Ca	K	Mg	
1	2	3	4	5	6	7
5·5	0·067	0·529	6·0	1·16	2·5	7·5

TABLE II

Organic carbon of the soil samples expressed as mg per 100 gms of soil

Treatments (Details given above)	1957-58		1958-59		1960-61	1962-63	1964-65		1965-66		Mean
	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	Kharif	Rabi	
1.	624	664	622	552	534	576	540	585	570	600	587
2.	595	589	559	597	534	570	510	525	600	585	566
3.	820	710	822	836	705	705	735	735	765	785	762
4.	755	707	839	799	661	660	681	690	735	750	728
5.	693	660	704	768	549	555	580	565	675	630	638
6.	660	801	859	831	679	655	606	710	750	810	736
7.	710	656	699	831	552	615	630	651	690	705	675
8.	760	616	768	722	549	640	630	550	650	720	661
9.	710	729	893	877	661	720	741	741	780	710	756
10.	640	784	859	831	697	650	705	705	735	765	737
11.	640	690	699	624	643	570	555	520	540	530	601
12.	680	651	768	831	649	600	765	765	766	710	719

TABLE III

Mean (10 years') yield of maize and wheat in maunds/acre

Treatments	Maize in mds/acre	Wheat in mds/acre
1.	5·92	5·64
2.	17·45	6·10
3.	19·46	14·75
4.	17·68	15·19
5.	19·04	15·71
6.	20·50	15·00
7.	22·10	15·95
8.	17·99	16·75
9.	18·19	13·99
10.	19·75	15·79
11.	22·63	17·89
12.	22·81	14·05

It is evident from the data given in Table 2 that organic carbon content of the control plot has varied from 534 mg to 664 mg per 100 gm of soil, the average of 10 seasons being 587 mg%. The mean yields of maize and wheat (mean of 10 years) from the control plot are 5·92 and 5·64 maunds per acre and from nitrogen alone treated plot are 17·45 and 6·19 maunds per acre respectively (Table 3). Perhaps, maize being a nitrogen loving crop has responded well to nitrogenous fertilizer even when applied singly. It is interesting to note that in spite of continuous cropping, the organic carbon of the control plot is maintained round about 580 mg%. According to Jenny and Raychaudhuri (1960)[5] the organic carbon content of cultivated humid region soils of India at 25°C (the mean annual temperature of Ranchi) ought to be 600 mg%.

The average organic carbon contents in the soil samples from N, NP and NPK treated plots are 566 638, and 661 mg% respectively (Table 2). The corresponding mean yields of maize are 17·45, 19·04 and 17·99 and of wheat 6·10, 15·71 and 16·75 maunds per acre (Table 3). These data indicate that phosphatic fertilizer has remarkably increased the yields (more in the case of wheat), while the potassic fertilizer over N and P has remained ineffective in case of maize, but has shown slight increas in the yields of wheat. The organic carbon content in these three treatments has therefore, followed the same trend as that of yields. The successive increase in yields may be due to residual effects of organic matter added by crops after harvest. Seasonal application of farm yard manure has resulted in a further increase in the organic carbon of the soil, the average of 10 seasons being 762 mg%.

The effect of applying inorganic fertilizers in manured plots on organic carbon reserve of the soil is evident if comparisons are made among the treatments 6 and 9; 7 and 10 (Table 3). The mean yields of maize in these treatments have varied between 18·19 and 22·10 and that of wheat between 13·99 and 15·95 maunds per acre. Thus, the effect of crop residues towards organic

matter addition to the soil may be taken to be equal in these cases. The observed differences in the organic carbon values are due to fertilizers applied to the manured plots. It is evident from the data that in general phosphatic fertilizer, when applied to manured plot (Treatments 6 and 7) shows a lower organic carbon reserve than the combined application of phosphatic and potassic fertilizers to such plots (Treatments 9 and 10). It seems that K has helped in conserving soil humus.

The long term effect of liming on the organic carbon reserves of the acid soil is also clear from Table 2. The mean organic carbon contents of the soil receiving NPK together with lime and no lime (Treatments 11 and 8) are 601 and 661 mg% respectively. The respective values with F.Y.M.+lime and no lime (Treatments 9 and 12) are 719 and 756 mg%. The data tend to show that liming is beneficial. It is likely that liming helps oxidation of organic carbon reserve of the soil. F.Y.M. when applied under limed conditions has shown a higher organic carbon compared to the NPK treated plot which has been similarly limed. Comparison made between the control and L+NPK treatment show that the organic carbon in the latter is during the last 4 seasons (1964-65 and 65-66), generally lower than that in the control plot, although the crop residues in the latter were in larger quantities. Liming in the latter has helped not only in the oxidation of the added crop residues but also of the native reserve of organic matter.

Summary

(i) The organic carbon content averaged over 10 seasons showed that nitrogenous fertilizer treatments produced lower organic carbon reserve than the control.

(ii) Potassic fertilizer did not increase yields of either maize or wheat, but it caused increase of the organic carbon content of the soil.

(iii) Fertilizers and manures applied to limed soil produced lower organic carbon reserve than the treatments receiving no lime. Lime was found to help oxidation of added organic matter.

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ELECTROMETRIC STUDY ON POLY-VANADATES OF ZIRCONIUM (IV)

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Vanadium (V) oxy-anions, which figure prominently in discussion of isopoly acids, exist in solution as four different stable species[1-3] viz. $(VO_4)^{3-}$, $(V_2O_7)^{4-}$, $(VO_3)^{1-}$ and $(V_{10}O_{27})^{4-}$ in the vicinity of pH,—12.0, 10.0, 7.0 and 4.5 respectively. The investigations on the reactions of these anions with heavy metal salts at different pH levels have been the subject of considerable interest in authors' laboratories due to their peculiar behaviour and have yielded significant results on poly-vanadates of several metals[4-8]. The literature on vanadates of zirconium is very scanty. A few old references are, however, available regarding the preliminary preparative studies of zirconium vanadates of uncertain compositions. Tanatar and Kurowsky[9] obtained zirconium oxy-chlorovanadate $m.ZrCl_4.n\ Zr_3(VO_4)_4.p.ZrO_2$ by mixing alkali vanadate and $ZrCl_4$. G. Peyronel[10] reported the formation of Zirconyl vanadate, $3\ ZrO_2.\ 2V_2O_5\text{-}9H_2O$ by heating $NH_4VO_3$and $ZrO(NO_3)_2$. A. Burdese and M. Barlvro[2] studied the binary system $ZrO_2\text{-}V_2O_5$ and reported the formation of ZrV_2O_7. In view of conflicting reports of earlier workers, it was considered of interest to study the formation and composition of zirconium vanadates, obtained by the interaction of zirconium sulphate and alkali vanadates at different H^+ ion concentrations by means of potentiometric and conductometric titrations.

Experimental

Anal. R (B.D.H.) reagents, V_2O_5, NaOH, $Zr(SO_4)_2$ and reagent grade HCl were used and their solutions prepared in air free conductivity water. Zirconium in zirconium sulphate was estimated as its pyro-phosphate[12].

A standard solution of sodium ortho-vanadate was prepared by digesting one mole of V_2O_5 in boiling solution of NaOH containing six moles of it. The alkali pyro and meta vanadate solutions were prepared by adding two and four moles of HCl respectively to a solution containing one mole of ortho-vanadate at 100°C.

E.M.F. measurements were carried out on a Cambridge null deflection type pH meter. A bright platinum foil was used as an indicator electrode in con-

junction with saturated calomel electrode, connected to the cell by agar-kCl bridge. The e.m.f. was noted after each addition of the titrant, in small increments which were further decreased in the region of the end point and plotted against the volume of the titrant added. The end points were determined from the sharp inflection in titration curves and maximum values of dE/dV.

Conductance measurements were carried out on an electronic eye indicator type conductometer. The conductance values after correction for dilution effect were plotted as a function of ml of titrant added and from the breaks in titration curves, the end points were determined.

Using different concentrations of reactants a series of e.m.f. and conductometric titrations were carried out both by the direct and the reverse methods (i.e. when $Zr(SO_4)_2$ solution from the microburette was added to alkali vanadate solution in the titration cell and vice-versa). Same strengths of solutions were employed in both the techniques for the sake of comparison of results. Titrations were also carried out in presence of varying concentrations of ethanol. Figures illustrating only direct potentiometric (Fig. 1) and conductometric (Fig. 2) titrations are given and the results showing also reverse titrations are summarised in Table I.

Results and Discussion

Saxena and co-workers[1-3] have shown that the addition of acid to alkali orthovanadate and NaOH to V_2O_5 solutions at room temperature causes the formation of various polyanions of uncertain composition, but when the solutions were heated after each addition of titrant, three different vanadates, pyro-($2Na_2O.V_2O_5$), meta ($Na_2O.V_2O_5$) and poly-($Na_2O.2.V_2O_5$) are formed. Hence it was considered of interest to ascertain whether similar salts of heavy metals may be precipitated as a result of double decomposition. The reaction between alkali vanadates and zirconium (IV) has, therefore, been studied by means of potentiometric and conductometric titrations with regard to the changes occurring in H^+-ion concentrations and compositions of the precipitate formed.

Ortho Vanadate Titrations : The pH of the $Zr(SO_4)_2$ and alkali ortho vanadate solutions was measured by a wide range glass electrode and found to be in the vicinity of 2·0 and 12.0 respectively. Fig. 1 illustrates the changes occurring in e.m.f. values during the reaction between Zr^{4+} and VO_4^{3-} ions. In the case of direct titrations (Fig. 1 -curve I), when zirconium salt from the microburette is added to ortho-vanadate solution in the titration cell, the e.m.f. of the solution gradually decreases at first; on the addition of 0.75 mole of $Zr(SO_4)_2$ per mole of ortho vanadate a sharp fall in e.m.f. is noted at the point where the molecular ratio of $ZrO_2 : V_2O_5$ is 3 : 4, suggesting the formation of an intermediate compound $3ZrO_2.2Na_2O.4V_2O_5$ in the pH range (9.0-9.5). On further addition of zirconium sulphate solution, a second more pronounced inflection is obtained at the molecular ratio of $ZrO_2 : V_2O_5$ as 3 : 2 corresponding to the formation of zirconium ortho-vanadate $3ZrO_2.2V_2O_5$ in the pH range

TABLE I

Summary of the results of E.M.F. and conductometric titrations

Molarity of solutions $3Na_2O.V_2O_5$: $Zr(SO_4)_2$		Equivalence points 'ml'				
		Cal.	observed from			
			Max. dE/dV ortho vanadate titration :—		Conductance	
			Direct titrations figs. 1 and 2—curve-I.			
M/100	M/10	3·75	1·85	3·75	1·80	3·75
M/200	M/25	4·70	2·35	4·70	2·4	4·75
M/500	M/40	3·0	1·5	3·0	1·45	3·0
M/750	M/50	4·0	1·95	4·10	1·90	4·10
			Reverse titrations.			
M/10	M/50	3·33	3·35		—	
M/15	M/80	3·12	3·10		—	
M/25	M/100	4·17	4·20		—	
M/60	M/200	5·0	5·0		—	
			Pyrovanadate titration:— Direct titrations figs. 1 and 2—curve II.			
$2Na_2O.V_2O_5$: $Zr(SO_4)_2$						
M/50	M/10	5·0	5·0		5·0	
M/125	M/20	4·0	4·0		4·0	
M/250	M/30	3·0	3·0		3·0	
M/400	M/40	2·5	2·45		2·5	
			Reverse titrations.			
M/10	M/50	5·0	5·0		5·0	
M/15	M/100	3·75	3·75		3·75	
M/20	M/150	3·33	3·30		3·35	
			Meta vanadate titrations:— Direct titrations figs. 1 and 2—curve III.			
$Na_2O.V_2O_5$: $Zr(SO_4)_2$						
M/50	M/10	3·0	3·0		2·95	
M/100	M/25	3·75	3·75		3·70	
M/300	M/50	2·5	2·45		2·5	
M/500	M/100	3·0	2·95		2·90	
			Reverse titrations.			
M/10	M/100	6·0	5·95		5·95	
M/10	M/150	4·0	4·0		4·0	
M/15	M/200	4·5	4·5		4·45	

(5.8-7.5). In the case of reverse titrations, the addition of ortho-vanadate solution to zirconium—sulphate solution causes an initial decrease in the e.m.f. values till about half the volume of titrant required for the complete precipitation of zirconium ortho-vanadate is added. With the progress of the reaction, e.m.f. begins to increase and a pronounced upward jump is obtained at the stoichiometic end point corresponding to the formation of zirconium ortho-vanadate.

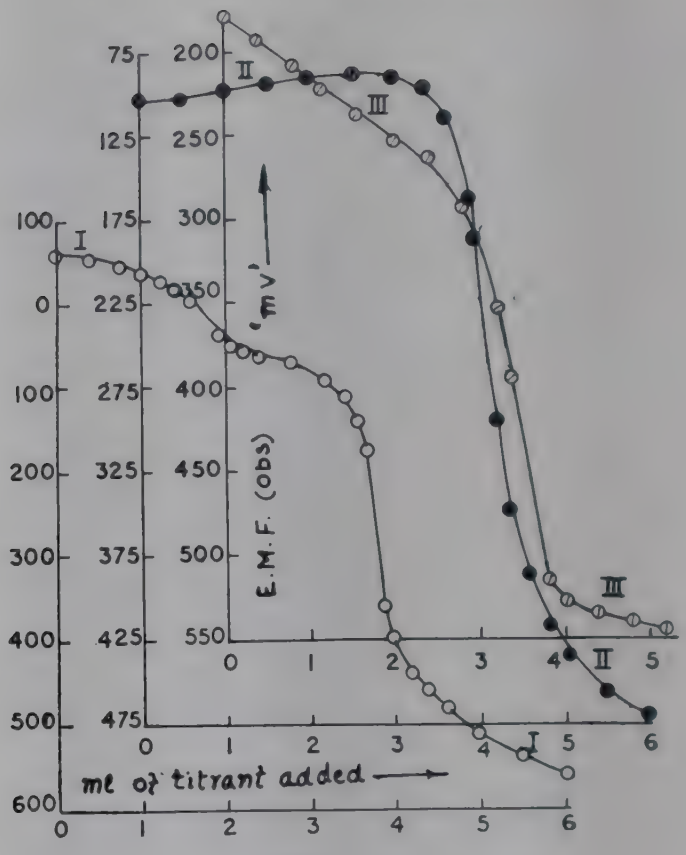

FIG. 1

Direct E.M.F. titrations between $Zr(SO_4)_2$ and Sodium vanadates.
Curve I. ml of M/10 $Zr(SO_4)_2$ added to 25 ml of M/100 $3Na_2O.V_2O_5$.
Curve II. ml of M/20 $Zr(SO_4)_2$ added to 25 ml of M/125 $2Na_2O.V_2O_5$.
Curve III. ml of M/25 $Zr(SO_4)_2$ added to 30 ml of M/100 $Na_2O.V_2O_5$.

Direct conductometric titration curves (Fig. 2 curve-I) yield two well defined breaks, confirming the formation of 3 $(ZrO_2.2Na_2O)$. $4V_2O_5$ and $3ZrO_2.2V_2O_5$ as indicated by potentiometric study. Reverse titration curves, however, do not exhibit any appreciable breaks.

From the preceding study it may be concluded that, when alkali ortho vanadate is present in excess, precipitation of zirconium ortho vanadate proceeds through the formation of an intermediate compound which, with the increase in Zr^{4+} content changes into ortho vanadate. The reaction equilibria can be stated as follows—

$$4(3Na_2O.V_2O_5)+3\ Zr(SO_4)_2=3\ (ZrO_2.2Na_2O).\ 4V_2O_5+6Na_2SO_4.$$
$$3\ (ZrO_2.2Na_2O).4V_2O_5+3\ Zr(SO_4)_2=2\ (3ZrO_2.2V_2O_5)+6Na_2SO_4.$$

Pyro-vanadate Titrations : Alkali pyro-vanadate solution was prepared as described earlier. In direct titrations, the plots of e.m.f. Vs. the volume of each aliquot (fig. 1, curve-II), are regular in form and exhibit well defined breaks at the point corresponding to the formation of zirconium pyro vanadate—$(ZrO_2.V_2O_5)$ in the pH range (5·5-4·0). The reverse titration also exhibits processes similar to those of ortho-vanadate and supports the formation of zirconium pyro-vanadate, which has been found to be quantitative and the titrations offer

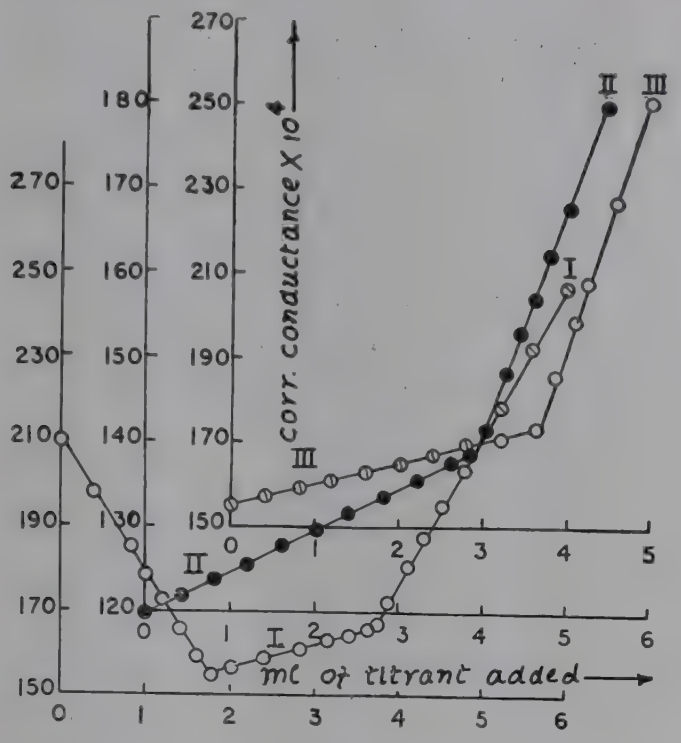

FIG. 2

Direct conductometric titrations between $Zr(SO_4)_2$ and Sodium vanadates.
Curve I. ml of M/10 $Zr(SO_4)_2$ added to 25 ml of M/100 $3Na_2O.V_2O_5$.
Curve II. ml of M/20 $Zr(SO_4)_2$ added to 25 ml of M/125 $2Na_2O.V_2O_5$.
Curve III. ml of M/25 $Zr(SO_4)_2$ added to 30 ml of M/100 $Na_2O.V_2O_5$.

a simple, rapid and accurate method of determining zirconium in solution at suitable concentrations and pH ranges. The reaction equilibria can be stated as—

$$3Na_2O.V_2O_5 + 2HCl = \underset{\text{(pyro)}}{2Na_2O.V_2O_5} + 2NaCl + H_2O.$$
(ortho)

$$2Na_2O.V_2O_5 + Zr(SO_4)_2 = ZrO_2.V_2O_5 + 2Na_2SO_4.$$

The course of this reaction was also followed by means of conductometric titrations. In direct titrations, when $Zr(SO_4)_2$ was used as titrant (Fig. 2, curve-II) the conductance values almost remained constant upto the addition of one mole of $Zr(SO_4)_2$ per mole of pyrovanadate, after which a sharp rise in conductance was noticed. The well defined breaks obtained from the titration curves suggest the formation of $ZrO^2.V_2O_5$. Reverse conductometric titration also exhibits well defined breaks at the stoichiometric end point, corresponding to the molar ratio of $ZrO_2 : V_2O_5$ as 1 : 1, suggesting the formation of zirconium pyrovanadate.

Meta-Vanadate Titrations : Sodium metavanadate solution was prepared by adding four moles of HCl to one mole of orthovanadate solution at 100°C and its reaction with Zr(IV) has been investigated by performing both direct and reverse potentiometric titrations. The titration curves (Fig. 1, curve-III) yield well defined breaks at the end point, indicating the formation of zirconium

metavanadate, ($ZrO_2.2V_2O_5$), in the pH range (4·0-3·0). The reaction can be represented as—

$$3Na_2O.V_2O_5 + 4HCl = Na_2O.V_2O_5 + 4NaCl + 2H_2O$$
(ortho) (meta)

$$2[Na_2O.V_2O_5] + Zr(SO_4)_2 = ZrO_2.2V_2O_5 + 2Na_2SO_4.$$

Conductometric titrations (Fig. 2, curve III) yield only one break corresponding to the formation of zirconium meta vanadate $ZrO_2.2V_2O_5$.

It is observed that the addition of alcohol does not appreciably change the end point or the nature of the curves, but the magnitude of the jump in e.m.f. increases in its presence. Potentiometric titration curves are regular in shape, dE/dV is strongly marked at the end point and the results are accurate and reproducible.

The investigations confirm the formation and precipitation of zirconium ortho-$3ZrO_2.2V_2O_5$, pyro-$ZrO_2.V_2O_5$ and meta-$ZrO_2.,2V_2O_5$ vanadates in the pH range (7.5·5.8), (5.5-4.0) and (4.0-3.0) respectively.

The formation and composition of zirconium poly-vanadates, obtained by the interaction of zirconium salt and different alkali vanadates (ortho, pyro and meta) have been investigated by means of potentiometric and conductometric titrations between the reactants at different pH levels in aqueous and aqueous-alcoholic media. The sharp breaks and inflections in titration curves provide definite evidence for the formation and precipitation of zirconium ortho, pyro and meta vanadates, having the compositions $3ZrO_2.2V_2O_5$, $ZrO_2.V_2O_5$ and $ZrO_2.2V_2O_5$ at pH ranges (7.5-5.8), (5.5-4.0) and (4.0-3.0), respectively. The precipitation of zirconium pyro-vandate is almost quantitative and the titrations offer a simple means of determining zirconium in solution at suitable concentrations and pH ranges.

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A NEW METHOD OF PREPARATION OF ETHYL ORTHOSILICATE

By

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(Received for publication on 18-5-68).

Ridge and Todd[1] have reviewed the preparation of alkyl orthosilicates and suggested a theory of formation of these compounds. In the above methods anhydrous alcohol has to be used, the preparation of which is time-consuming. During our recent investigations on the preparation of various metal alkoxides a simple method for their preparation has been discovered and in this method the alcohol produced in the reaction itself is used for the preparation of metal alkoxides.

It is well known that most of the alkyl esters of fatty acids react with ammonia with the formation of corresponding alcohol and acidamide. This property of the esters has been utilised for the preparation of various metal alkoxides. This communication however deals with the preparation of silicon tetra ethoxide by the interaction of silicon tetrachloride in benzene with ethyl formate, ethyl acetate and diethyl oxalate in the presence of dry ammonia.

All-glass apparatus with quickfit interchangeable joints was used and special precautions were taken to exclude moisture. Commercial silicon tetrachloride was purified by the fractional distillation and fractions boiling at 56—58°C were collected and used. Pure benzene was distilled over sodium and further dried by azeotropic distillation with alcohol. Ethyl formate, ethyl acetate and diethyl oxalate were dried over anhydrous magnesium sulphate before fractionation.

Preparation of silicon tetra-ethoxide :

Through dry benzene containing silicon tetrachloride and ester (quantities given in Table I) placed in a two necked one litre flask fitted with a reflux

TABLE I

Analytical data of ethyl orthosilicate

Ester	Ester taken (g.)	$SiCl_4$ taken (g.)	Benzene taken (ml.)	Yield %	Analysis Found % Si	Analysis Found % OEt
Ethyl formate ...	40	3·5	400	75-78	13·25	86·30
Ethyl acetate ...	35	3·0	350	70-72	13·22	86·42
Diethyl oxalate ...	25	2·5	300	68-70	13·40	86·25

Calc. for $Si(OC_2H_5)_4$: Si, 13·5; OEt. 86·5%

condenser, dry ammonia was passed. As the reaction was exothermic in all cases, ice bath was used to prevent the loss of benzene and the lower esters. On the completion of the reaction and after the removal of dissolved ammonia and benzene under reduced pressure, silicon ethoxide was obtained by distillation at 68°C/18 mm.

Silica in the ethoxide was estimated as SiO_2 while percentage of ethoxy group was found by chromic acid method[2, 3].

From the analytical data (given in Table I) the composition of the compound in all the cases studied above corresponds to $Si(OC_2H_5)_4$.

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A NEW APPROACH TO THE EVALUATION OF THE EFFICACY OF m-XYLOHYDROQUINONE AS A CANCER CHEMOTHERAPEUTIC AGENT

By

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In the evaluation of the efficacy of cancer chemotherapeutic agent, the generally accepted methods consist of tissue cultures or direct observation of the tumour, grafted into the susceptible strain of mice or on some mutant bacterial strains or radiobiological studies with tracer elements. The divergence regarding the method of study of the mode of action in the case of m-xylohydroquinone consists of physico-chemical study with spectrophotometer for the determination of the depolymerization of D.N.A. and also the blocking of the glycolytic cycle, the path of energy supply of the tumour cells.

During cell multiplication, the D.N.A. might be interfered with either by esterification[1] of the phosphate groups in the D.N.A. or by N-alkylation[2] of its constituent purine bases. D.N.A. activity might also be interrupted by ionic radiation which causes depolymerization of D.N.A. and brings about arrest at metaphase[3, 4, 5]. Also for D.N.A. synthesis, sulphydril[6] grou pof enzymes and folinic acid[7] are essential. By blocking these two substances, D.N.A. synthesis might be prevented.

Secondly, the multiplication of the cancer cells might be affected by interfering with the path of energy supply. According to Warburg[8] "shifting of the metabolism to the anaerobic state is the main biochemical difference between the tumour and normal cell. Adrenal cortical steroid, by opposing insulin activity inhibits the phosphorylation of glucose with A.T.P. by restraining hexokinase activity.[9] This has been utilized in the treatment of cancer cases by A.C.T.H. or prednisolone. A similar effect might also be achieved by blocking the glycolytic cycle at the stage of glucose-6-phosphate. m-xylohydroquinone has been observed to bring about both these effects, namely depolymerization of D.N.A. and the blockage of the glycolytic cycle at the stage of glucse-6-phosphate.

Experimental

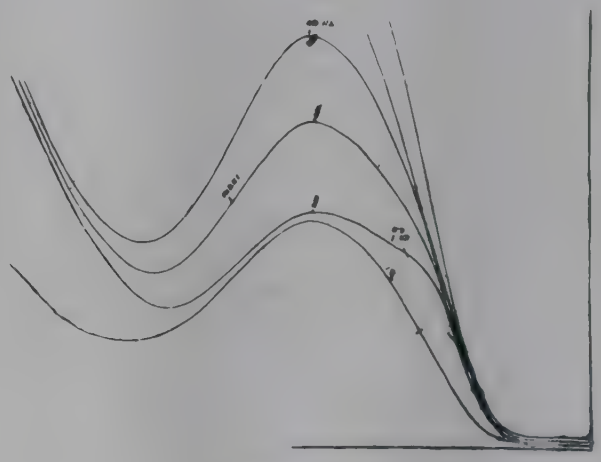
m-xylohydroquinone sol: m-xylohydroquinone was recrystallized thrice from small volumes of pure alcohol and dried in vacuo. 4.12 mg of the substance

was dissolved in 250 ml deoxygenated water, prepared by passing a stream of nitrogen gas through the water.

(Concentration :—1.203×10^{-4}M.).

D.N.A. Solution: 1.94 mg D.N.A. (Sigma) was dissolved in 25 ml deoxygenated water.

Glucose-6-Phosphate Solution:—8.2 mg of glucose-6-phosphate (barium salt) was taken in a centrifuge tube and was dissolved in 10 ml of deoxygenated water. To the solution about 4 mg of sodium sulphate (G.R.E. Merck.) was added. $PaSO_4$ thus formed was removed by Centrifuging. The sodium salt of glucose-6-phosphate thus formed was transferred to 100 ml measuring flask and made upto volume. (Concentration of glucose-6-phosphate, sodium salt—2.75×10^{-4}.).

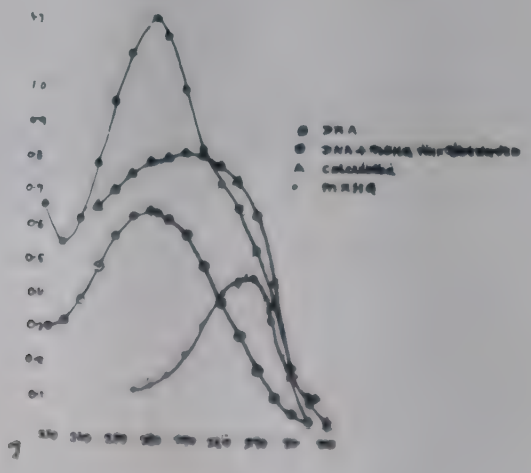


GRAPH—I

Interaction of D.N.A. and m-Xylohydroquinone:

Three different mixtures of the above solutions of D.N.A. and m-xylohydroquinone were prepared in different proportions and marked (1), (2) & (3).

(1)	5 ml of m-xylohydroquinone sol and	5 ml of D.N.A. sol	1	: 1
(2)	5 ml of ,,	10 ml of ,,	1	: 2
(3)	10 ml of ,,	5 ml of ,,	2	: 1



GRAPH—II

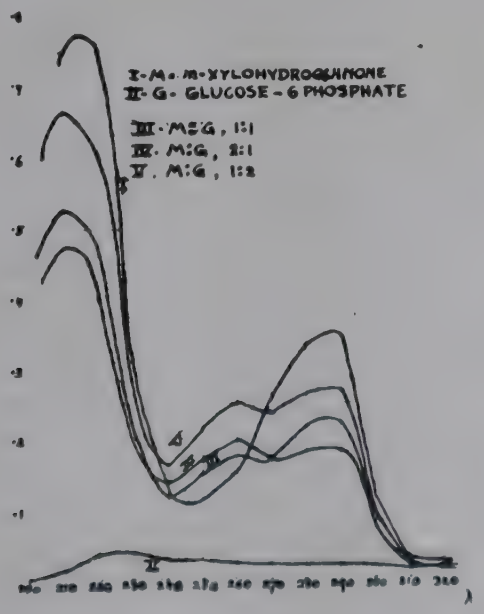
Spectra were taken in a Backman Recording Spectrophotometer with 1 cm of Quartz cell. They are reproduced in graph I. In graph II the observed curve of mixture 1 : 2 and the calculated curve were found to intersect indicating interaction.

Interaction of Glucose-6-Phosphate and m-Xylohydroquinone :

Three different mixture of the above solutions of m-xylohydroquinone and glucose-6-phosphate (sodium salt) were prepared in different proportions and marked III, IV & V.

I. The pure m-xylohydroquinone soln.
II. The pure glucose-6-phosphate (sodium salt) soln.
III. 5 ml of m-xylohydroquinone sol & 2.5 ml of glucose-6-phosphate sol 1 : 1
IV. 8 ml of ,, 2.0 ml of ,, 2 : 1
V. 5 ml of ,, 5.0 ml of ,, 1 : 2

The absorption densities of these five solutions were determined in a Hilger U.V. Spectrophotometer using 1 cm. quartz cell. They are reproduced in graph III.



GRAPH—III

A solution of fructose was prepared (1.88 mg in 100 ml of deoxygenated water) and a mixture of this fructose solution with m-xylohydroquinone solution was prepared in different proportions. Their absorption densities were determined in Beckman Recording Spectrophotometer. There was no sign of any interaction. This graph has not been included here.

Result and Discussion

From the foregoing experimental results it is evident that m-xylohydroquinone interacts with D.N.A. The observed and the calculated curves of the absorption densities intersect and also the absorption density of the same concentration of D.N.A. is enhanced due to its interaction with m-xylohydroquinone. Kunitz observed that when D.N.A. is depolymerized, the absorption density at 260 mμ is enhanced. This suggests that m-xylohydroquinone depolymerizes D.N.A. This depolymerization effect was observed in a comperative study of the antimitotic effects of X-ray irradiation and γ-radiation from radium berrylium and also m-xylohydroquinone[11] and another chemical, 2 :6 dimethylphenoxyaceticacid. This effect seems to be due to semiquinone[12] which is an intermediate between hydroquinone and quinone of this redox system. This semiquinone is a very potent single radical and can either donate or accept hydrogen according to the condition of the substrate. The semiquinone may remove the hydrogen bonds of D.N.A. and/or it may inactivate the sulphhydril group of enzymes essential for D.N.A. synthesis.

Secondly, it was observed in the spectrophotometric studies that glucose-6-phosphate is practically transparent whereas m-xylohydroquinone has an absorption maxima at 290 mμ but the mixture indicates the formation of a complex by the appearance of a separate absorption maxima at .60 mμ. It was previously observed that m-xylohydroquinone interacts with hexose and glucose and not with a-methylglucopyraniside.[13] Tentative conclusion is that m-xylohydroquinone interacts with the aldehyde group at C_1 of the glucose structure. Fructose has no aldehyde group at C_1 and as such no interaction could be observed with m-xylohydroquinone. From these observations it may be surmised that the blocking of C_1 position of glucose-6-phosphate by m-xylohydroquinone prevents its further conversion into fructose-6-phosphate and consequently the glycolytic metabolic path is blocked.

These experimental studies lend support to the encouraging results of clinical trials on a large number of cancer cases with m-xylohydroquinone. From these observations, both clinical and experimental, m-xylohydroquinone seems to be a promising chemotherapeutic agent in the treatment of cancer. It may act both ways, namely depolymerization of D.N.A. and also bockage of the metabolic path of the cancer cells. Unlike other commonly used antimitotic agents, it is practically non-toxic and can be administered in larger doses and over a prolonged period.

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